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Combined inhibitory effect of milk fat and lactose for inactivation of foodborne pathogens by ohmic heating

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ABSTRACT

Numerous dairy products are produced with reduced fat and/or lactose content as consumer demand for foods of modified nutritional content has increased recently. The combined inhibitory effect of milk fat and lactose on the inactivation of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* by ohmic heating was investigated in the present study. Response surface methodology with central composite design was used to analyze the inactivation of pathogens and develop a predictive model. Both lactose and fat had an inhibitory effect on the inactivation of all three pathogens by ohmic heating. Inactivation of *E. coli* O157:H7 has a quadratic relationship with lactose and fat, whereas the cross product of treatment time with fat or lactose has a significant effect on the inactivation of *S.* Typhimurium and L. *monocytogenes*. The developed model predicted the inactivation of all three pathogens well within the range of experimental conditions. Color change and lipid oxidation were not observed following ohmic heating, while pH values slightly decreased. Therefore, treatment conditions of ohmic heating should be decided carefully considering the lactose and fat content when using this method to pasteurize milk products.

1. Introduction

Foodborne illnesses have increasingly been reported worldwide, especially in developing countries due to lack of food safety management. Consuming food containing physical, chemical, and biological hazards can result in foodborne illness, hospitalizations, and deaths. Pathogenic bacteria are a major cause of hospitalizations, which account for 64% of hospital cases caused by contaminated food eaten in the United States (Scallan et al., 2011). In particular, *Escherichia coli* O157:H7, *Salmonella enterica* Serovar Typhimurium, and *Listeria monocytogenes* are representative foodborne pathogens which cause hemolytic uremic syndrome, salmonellosis, and epidemic listeriosis, respectively. Several preservation methods, including physical, chemical, and biological treatments, have been developed to control these foodborne pathogens. Even though chemical treatments have a powerful antibacterial effect, consumers avoid chemically treated food due to the possibility of toxicity. Biological treatments such as bacteriophage application are being investigated nowadays, but the host range is too narrow to apply this method to food production (Hudson et al., 2013). Therefore, physical treatments are still used widely to inactivate foodborne pathogens.

Physical treatments can be divided into thermal and non-thermal treatments. Non-thermal treatments such as pulsed electric field, LED-UV, and cold plasma have been investigated recently for inactivation of foodborne pathogens (Shin, Kim, Kim, & Kang, 2016; Timmermans et al., 2014; Yong et al., 2015). Even though quality degradation of food can be minimized by non-thermal treatment, only the food surfaces can be treated with interventions such as LED-UV and cold plasma. Quality degradation by severe heat is a major limitation of thermal treatment, which has long been used for food processing. Therefore, alternative thermal treatments such as 915 MHz microwave, radio frequency, and ohmic heating have been developed. Rapid heating by these alternative thermal treatments reduces quality degradation while inactivating foodborne pathogens (Jeong & Kang, 2014; Song &

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Kang, 2016). In particular, ohmic heating has an advantage in heating uniformity compared to 915 MHz microwave and radio frequency heating (Kim, Sung, et al., 2016).

Inactivation of foodborne pathogens by ohmic heating has been investigated recently (Kim & Kang, 2017; Kim, Choi, & Kang, 2017). The efficacy of ohmic heating is affected by intrinsic and extrinsic factors. Extrinsic factors such as voltage and frequency have a significant effect on the inactivation of pathogens. For example, accelerated heating rate by means of increased voltage and frequency results in rapid inactivation of foodborne pathogens (Baysal & Icier, 2010; Lee, Ryu, & Kang, 2013). Reduction of pathogens is also significantly affected by intrinsic factors. In particular, nutritive components such as fats, protein, and carbohydrates not only change the electrical conductivity of food but also have a protective effect on the inactivation of pathogens (Kim & Kang, 2015).

Consumer demand for foods of modified nutritional content has been increasing recently, and accordingly, such foods have appeared in the market place. Particularly, various milk products are being produced which have reduced fat and/or lactose content. Fat content of whole milk, low fat milk, and skimmed milk are 3–4, 1–2, and 0–0.5%, respectively. Low fat or skimmed milk is preferred by some consumers who worry about obesity. Lactose content is about 4–5% in whole milk, which can cause lactose indigestion in lactose-intolerant consumers (Scrimshaw & Murray, 1988). Therefore, lactose-free milk is preferred by some consumers who have trouble digesting this sugar. The level of fat and/or lactose can influence inactivation of foodborne pathogens by thermal or non-thermal treatments. The inactivation levels of *S.* Typhimurium DT 104 and *Listeria innocua* decreased with increasing fat content when treated with a shaking water bath (Juneja & Eblen, 2000) and oil bath (Bermúdez-Aguirre & Barbosa-Cánovas, 2008), respectively. Ramaswamy, Jin, and Zhu (2009) reported that casein and lactose are important factors affecting the baro-protection of *E. coli* in milk during high-pressure treatment. Even though ohmic heating has been used to process milk products, to the best of our knowledge, research about the combined effect of milk fat and lactose on the inactivation of pathogens is very limited.

In the present study, the combined effect of milk fat and lactose on the inactivation of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* was investigated. First, reductions of the three pathogens subjected to ohmic heating in milk of various fat and lactose content were analyzed by response surface methodology (RSM). Secondly, a predicted model developed by RSM was verified within the range used in the experiments. Finally, quality aspects including pH, color, and lipid oxidation were assessed.

2. Materials and methods

2.1. Bacterial cultures and cell suspension

Three strains each of *E. coli* O157:H7 (ATCC 35150, ATCC 43889, ATCC 43890), *S.* Typhimurium (ATCC 19585, ATCC 43971, DT 104), and *L. monocytogenes* (ATCC 19111, ATCC 19115, ATCC 15313) were obtained from the bacteria culture collection of Seoul National University (Seoul, Korea). Stock and working cultures were prepared according to a previously described method (Kim & Kang, 2015a). A single colony cultivated from frozen stocks on tryptic soy agar (TSA; Difco, Becton, Dickinson, Sparks, MD) was inoculated into 5 ml of tryptic soy broth (TSB; Difco, Becton, Dickinson, Sparks, MD), incubated at 37 °C for 24 h, collected by centrifugation at 4000 × g for 20 min at 4 °C, and washed three times with 0.2% peptone water (PW; Bacto, Becton, Dickinson, Sparks, MD). The final pellets were resuspended in 0.2% PW. Afterwards, suspended pellets of the three pathogens were combined to comprise a mixed culture cocktail containing approximately equal numbers of cells of each strain of *E. coli* O157:H7 (10^9 CFU/ml), *S.* Typhimurium (10^9 CFU/ml), and *L. monocytogenes* (10^8 CFU/ml).

2.2. Sample preparation and inoculation

Pasteurized lactose free and low fat (1.5%) milk (pH 6.9) and sterilized cream containing 37% fat and emulsifier were purchased at a local grocery store (Seoul, South Korea). Milk and cream were stored under refrigeration (~4.0 °C) until used for experiments. Cream and lactose (Difco) were added to milk to achieve fat contents of 1.5, 2.5, 3.5, 4.5, or 5.5% and lactose content of 0, 1, 2, 3, or 4%. Fat and lactose content were calculated on the basis of manufacturer's declarations. Samples were mixed using a magnetic stirrer and stir bar. Mixed-culture cocktail (0.2 ml) was inoculated into 50 mL of prepared sample.

2.3. Experimental design

The effect of lactose, fat, and treatment time on the inactivation of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* was identified using RSM. Lactose and fat levels ranged from 0 to 4% and 1.5–5.5%, respectively, depending on food products. Treatment time was determined to be 80–120 s based on our preliminary studies. A 3-factor Central Composite Design (CCD) was used and five levels for each factor were coded as −2, −1, 0, +1, +2, respectively (Table 1). The 16 experiments were performed in random order.

2.4. Ohmic heating treatment

Ohmic heating treatments were carried out in a previously descried apparatus (Kim & Kang, 2015b). The ohmic heating system consisted of a function generator (catalog number 33210A; Agilent Technologies, Palo Alto, CA), a precision power amplifier (catalog number 4510; NF Corp., Yokohama, Japan), a two-channel digital-storage oscilloscope (catalog number TDS2001C; Tektronix, Inc., Beaverton, CO), a data logger (catalog number 34970A; Agilent Technologies), and an ohmic heating chamber. The distance between the two electrodes was 4 cm, and the cross-sectional area was 60 cm^2. Prepared and inoculated samples were subjected to pulsed ohmic heating (0.3 duty ratio, 10 kHz) with fixed electric strength of 18.2 V_{rms}/cm. Samples were taken after each treatment and populations of surviving microorganisms were enumerated.

2.5. Bacterial enumeration

For microbial enumeration, each treated 50 mL sample was immediately transferred into a sterile stomacher bag (Labplas, Inc., Sainte-Julie, Quebec, Canada) containing 100 ml of sterile 0.2% peptone water (4 °C) and homogenized for 2 min using a stomacher (Easy Mix; AES Chemunex, Rennes, France). After homogenization, 1 ml samples were 10-fold serially diluted with 9 ml of sterile 0.2% peptone water and 0.1 ml of stomached or diluted samples were spread-plated onto each selective medium. Sorbitol MacConkey (SMAC) agar (Difco), xylose lysine deoxycholate (XLD) agar (Difco),

Table 1
Variables and levels used for the central composite design.

Xi	Independent variables	Levels				
		−2	−1	0	+1	+2
X_1	Lactose content (%)	0	1	2	3	4
X_2	Fat content (%)	1.5	2.5	3.5	4.5	5.5
X_3	Time (s)	80	90	100	110	120